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Short communication

Peripheral administration of interleukin-13 reverses inflammatory macrophage and tactile allodynia in mice with partial sciatic nerve ligation

Norikazu Kiguchi*, Haruka Sakaguchi, Yui Kadowaki, Fumihiro Saika, Yohji Fukazawa, Shinsuke Matsuzaki, Shiroh Kishioka

Department of Pharmacology, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-0012, Japan

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ABSTRACT

Inflammatory macrophages play a fundamental role in neuropathic pain. In this study, we demonstrate the effects of peripheral interleukin-13 (IL-13) on neuropathic pain after partial sciatic nerve (SCN) ligation (PSL) in mice. IL-13 receptor α1 was upregulated in accumulating macrophages in the injured SCN after PSL. Treatment with IL-13 reduced inflammatory macrophage-dominant molecules and increased suppressive macrophage-dominant molecules in cultured lipopolysaccharide-stimulated peritoneal macrophages and *ex vivo* SCN subjected to PSL. Moreover, the perineural administration of IL-13 relieved tactile allodynia after PSL. These results suggest that IL-13 reverses inflammatory macrophage-dependent neuropathic pain *via* a phenotype shift toward suppressive macrophages.

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Macrophages are categorized into several distinct phenotypes based on the expression patterns of inflammatory molecules. The classical activation of M1-polarized macrophages is promoted by bacterial lipopolysaccharide (LPS) or Th1 cytokines facilitating inflammation, whereas alternative activation of M2-polarized macrophages is induced by Th2 cytokines (i.e., interleukin-4 (IL-4) and IL-13), which suppress inflammation (1,2). Thus, the balance between M1/M2 macrophage polarization is a fundamental component in the regulation of inflammation.

Neuronal damage often results in neuropathic pain, characterized by prolonged abnormal pain sensations such as tactile allodynia (3,4). Growing evidence suggests that chronic neuroinflammation caused by inflammatory molecules underlies neuropathic pain (5,6). Notably, M1 macrophages accumulate in injured nerves, and produce various pro-inflammatory molecules leading to peripheral neuroinflammation (7). We also previously showed that macrophage suppressive agents or inhibitors for M1 macrophage-derived pro-inflammatory cytokines (e.g., IL-1β) and chemokines (e.g., CC-chemokine ligand 3 (CCL3)) prevented neuropathic pain in mice (8–10). Given that M1 macrophages are critical for neuropathic pain, it is important to understand the relationship between the pharmacological regulation of M1/M2 polarization and neuropathic pain pathogenesis.

Recently, we reported that the local administration of IL-4 around injured peripheral nerves ameliorated neuropathic pain in mice after sciatic nerve (SCN) injury, in line with the phenotype shift from M1 to M2 macrophages (11). Because IL-4 receptor subunit α (IL-4Rα) signaling activates STAT6, a key transcription factor for M2 molecules, through a heteromeric complex with γ-chain or IL-13 receptor subunit α1 (IL-13Rα1) (12), IL-4 as well as IL-13 may exert suppressive effects on M1 macrophage-related inflammation *via* a common receptor, IL-4Rα (13). In this study, we demonstrate that the perineural administration of IL-13 reverses M1 macrophage polarization and relieves neuropathic pain after nerve injury in mice.

All experimental procedures were approved by the Animal Research Committee of Wakayama Medical University. Male ICR mice aged 4–5 weeks (SLC, Hamamatsu, Japan) were housed in plastic cages, and provided with water and food *ad libitum*. Mice were subjected to partial sciatic nerve ligation (PSL) according to a well-established method (14). Under isoflurane anesthesia, the SCN was exposed through a small skin incision on the left side (ipsilateral). Approximately one-third of the SCN was tightly ligated

* Corresponding author. Fax: +81 73 446 3806.
E-mail address: kiguchi@wakayama-med.ac.jp (N. Kiguchi).
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with a silk suture, and the incision was then closed by suturing. The SCN on the right side (contralateral) was exposed without ligation as a sham control.

Recombinant mouse IL-13 (R&D Systems, Minneapolis, MN) and LPS (Sigma–Aldrich, Tokyo, Japan) were dissolved in sterile phosphate-buffered saline (PBS). For the *in vivo* experiment, 10 μl of IL-13 (100 ng) was perineurally injected without a skin incision into the region surrounding the SCN using a microsyringe fitted with a 30-gauge needle under isoflurane anesthesia (8,11).

To collect peritoneal macrophages (PMs) from naïve mice, an incision was made in the peritoneal membrane and 5 ml of chilled sterile PBS containing 1% penicillin-streptomycin (P/S) was slowly injected into the peritoneal cavity (10). Collected PMs after flushing were cultured in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% P/S. *Ex vivo* SCN culture was performed as previously described (11). Mice subjected to PSL or sham were euthanized on day 7, and a 10-mm length of fresh SCN was isolated and immersed in chilled DMEM containing 1% P/S. PMs and SCN were incubated in DMEM without adding FBS at 37 °C for 24 h.

RNA was extracted from freshly isolated SCN, PMs, or *ex vivo* cultured SCN using TRIzol reagent (Invitrogen, Carlsbad, CA). Purified total RNA (1 μg) was used to synthesize cDNA, and 10 ng of cDNA was used as a template for quantitative real-time PCR using the KAPA SYBR FAST qPCR Kit (Kapa Biosystems, Boston, MA). Primer sequences (Operon Biotechnology, Tokyo, Japan) are shown in Supplementary Table 1. The fluorescent intensity of the intercalated SYBR Green was measured and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

The fixed frozen SCN was cut longitudinally into 10-μm-thick sections and mounted on a glass slide. Sections were blocked with 4% bovine serum albumin for 2 h and incubated at 4 °C overnight with primary antibodies against IL-13Rα1 (Santa Cruz Biotechnology, Santa Cruz, CA) or F4/80 (Cederlane, Burlington, Canada). Sections were washed and incubated with secondary antibodies conjugated with fluorescent dyes (Alexa-488 or Alexa-594; Invitrogen). Fluorescence was detected using a confocal laser scanning microscope.

Tactile allodynia was assessed by the von Frey test as previously described (8,11). Mice were allowed to adapt for 2–3 h on a 5 × 5-mm wire mesh grid floor and covered with an opaque cup. The 0.07 g von Frey filament (Neuroscience, Tokyo, Japan) was applied to the middle of the plantar surface of the hind paw through the bottom of the mesh floor. Withdrawal responses to tactile stimulation were measured 10 times for each hind paw, and tactile allodynia was defined by the number of withdrawal responses.

All data are presented as mean ± SEM. Statistical analysis was performed using one-way analysis of variance followed by Tukey's multiple comparison test. Significance was established at $P < 0.05$.

The mRNA expression of IL-13 in the injured SCN was downregulated on day 3, followed by upregulation on day 7 after PSL compared to sham control (Fig. 1A). On the other hand, mRNA expression of IL-13Rα1 in the injured SCN was upregulated on days 3, 7, and 14 after PSL (Fig. 1B). Immunohistochemistry showed that IL-13Rα1 was increased in the injured SCN on day 7 after PSL, and that this localized to most F4/80-positive macrophages (Fig. 1C).

In the PMs, exposure to LPS for 24 h increased the mRNA expression of M1-dominant molecules IL-1β and CCL3, and this expression was reduced by co-incubation with IL-13. mRNA expression of M2-dominant molecules arginase 1 (Arg1) and CD206 was increased by IL-13 treatment for 24 h with or without LPS (Fig. 2A). Next, the direct effects of IL-13 on accumulating macrophages in the injured SCN were determined by *ex vivo* SCN cultures. The mRNA expression of M1 molecules was greater in PSL-operated SCN cultures than in sham-operated controls. Expression in PSL-operated SCN cultures was reduced by IL-13 treatment for 24 h, but IL-13 had no effect on controls. The mRNA expression of CD206 in PSL-operated SCN was increased by IL-13, while upregulated Arg1 in PSL-operated SCN was not affected by IL-13. IL-13 also upregulated the expression of CD206 and Arg1 in sham-operated SCN (Fig. 2B).

To assess the pharmacological effects of IL-13 on PSL-induced tactile allodynia, IL-13 was perineurally administered on days 7, 9, 11, and 13 after PSL, and tactile allodynia was measured on day 14. PSL-induced tactile allodynia in the ipsilateral paw was significantly reversed by IL-13, but IL-13 had no effect on contralateral paw control (Fig. 3).

We found the upregulation of IL-13Rα1 in accumulating macrophages for at least 2 weeks after PSL, consistent with the upregulation of IL-4Rα that we reported previously (11). Therefore, we hypothesized that functional receptor complex of IL-13Rα1 and IL-4Rα might increase in accumulating M1 macrophages indicating possible plasticity for a phenotype shift from M1 to M2 macrophages. This is supported by the results that IL-13 reduced expression of M1 molecules (IL-1β and CCL3) and increased M2 molecule expression (Arg1 and CD206) in LPS-stimulated M1 macrophages, an *in vitro* model of accumulating M1 macrophages in the injured SCN. Moreover, IL-13-induced upregulation of M2

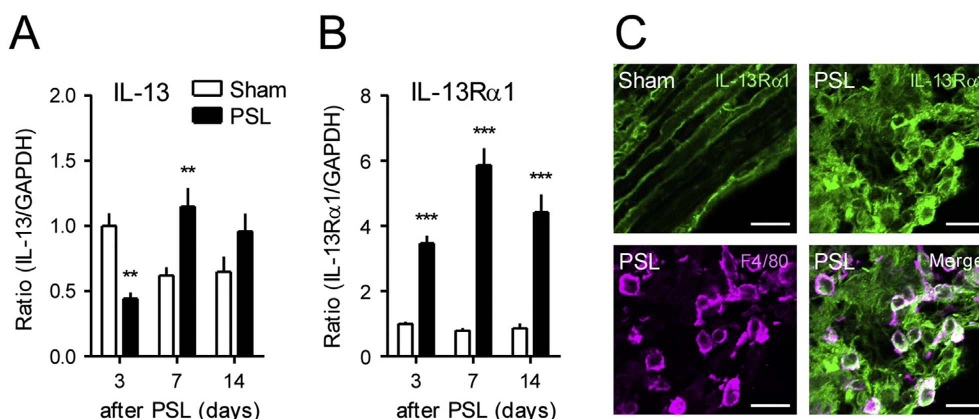

Fig. 1. Upregulation of interleukin-13 receptor α1 (IL-13Rα1) in accumulating macrophages. Mice were subjected to partial sciatic nerve (SCN) ligation (PSL) or sham surgery, and the SCN was collected. The time course of IL-13 (A) and IL-13Rα1 (B) mRNA expression in the injured SCN after PSL was evaluated by RT-qPCR. Data are presented as the mean ± SEM of 5–6 mice. ***$P < 0.001$ vs sham control. (C) Localization of IL-13Rα1 protein on accumulating F4/80$^+$ macrophages in the injured SCN on day 7 after PSL was visualized by immunohistochemistry. Representative micrographs in longitudinal sections are shown. Scale bars = 20 μm.

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